THE RELATIONSHIP OF TEMPERATURE TO THE LARVAL DEVELOPMENT OF NASSARIUS OBSOLETUS (GASTROPODA) [1, 2]

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Temperature has long been suggested as an important factor regulating the developmental rate, length of pelagic life, and mortality of larvae from benthic marine invertebrate organisms. It is known, for example, that the rate of early cleavage, within certain limits, is related directly to water temperature (*vide* Costello *et al.*, 1957). There have been a number of attempts by marine biologists, especially with species of economic value, to relate the success of settlement during any specific year to the sea water temperature at the time of larval development. Among bivalve mollusks, the oysters *Crassostrea virginica* Gmelin and *Ostrea edulis* L. and the clam *Venus mercenaria* L. have particularly been studied (*e.g.*, Needler, 1940; Medcof, 1939; Korringa, 1952; Carriker, 1961, pp. 212–213).

Seno, Hori and Kusakabe (1926) determined the effect of temperature on the early development of *Ostrea gigas* from the time of fertilization to the early shelled larva. Clark (1935) examined the effect of reduced temperature on the early development of *Crassostrea virginica.* Not until the development of adequate techniques for growing larvae in mass culture from fertilization to settlement (Allen and Nelson, 1911; Bruce *et al.*, 1940) has it been possible to examine experimentally the relationship of temperature to the development of molluscan larvae. Loosanoff *et al.* (1951) and Loosanoff (1959) were the first to demonstrate successfully in the laboratory the role of temperature throughout the entire period of pelagic larval development of the bivalve mollusk, *Venus mercenaria.* Subsequently studies of comparable detail have been made by Walne (1958) with *Ostrea edulis;* by Davis and Calabrese (1964) with *Crassostrea virginica;* by Stickney (1964) with *Mya arenaria* L.; and by Bayne (1965) with *Mytilus edulis* L. The very interesting research on gastropod larvae by Lebour (1937) did not include experimental work using mass culture techniques, as her primary concern was the identification and description of veligers from the plankton. Similarly, Thorson (1946, 1950) has followed the development of gastropod larvae by examining plankton tows periodically taken from the Øresund, but he has not attempted to undertake laboratory culture work as a means of understanding the relative importance of environmental factors on pelagic larval development. It is the purpose of this paper to describe such a laboratory study using the common marine intertidal prosobranch gastropod *Nassarius obsoletus* Say.

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Nassarius obsoletus inhabits marine and estuarine intertidal flats from Chaleur Bay in the Gulf of St. Lawrence to Cape Kennedy (Canaveral) in northern Florida. Although the early cleavage stages of development are well known to embryologists (*vide*: Clement, 1962; Thompson, 1955), the later planktotrophic veliger larvae were not described until recently (Scheltema, 1962a). Experimental studies have determined the role of salinity in larval survival and development (Scheltema, 1962b, 1965). Some mechanisms which control the length of pelagic life and the delay of metamorphosis are also known from previous experiments (Scheltema, 1961). Aspects of the ecology of the adults have been discussed by Dimon (1905), Jenner (1956a, 1957), and Scheltema (1964).

This study is divided into two parts: (1) the relationship of temperature to the rate of early embryological development within the egg capsule, as indicated by the time required for emergence of veliger larvae into the sea; (2) the relationship of temperature to growth and length of planktonic larval life. Before giving an account of the experimental work, however, I shall describe briefly the reproductive habits of *N. obsoletus,* as these have not previously been recorded in any detail.

Reproduction and Spawning

The onset of spawning in *Nassarius obsoletus* differs with latitude and is directly related to sea water temperature. As the species inhabits an environment where temperature can be highly variable over short periods, the exact timing of reproduction is never very precise. It can be shown, for example, that at Beaufort, North Carolina, the water temperature on the intertidal flats in early February may differ as much at 5° C. between high and low tide; a change from 13° to 17° C. has been recorded in the area on the flats where females of *N. obsoletus* occur. At Barnstable Harbor, Cape Cod, Massachusetts, the low-water temperature on the flats inhabited by *N. obsoletus* increases abruptly in a period of about two to three weeks from 13° C. in mid-May to 23° C. in early June. That the females respond to elevation of water temperature by spawning can easily be shown by bringing snails into the laboratory during mid-winter months. Under such conditions, when the animals are fed, spawning commences within a week. Copulation occurs during the same period as spawning.

Under natural conditions the process of gametogenesis is completed long before the normal time for spawning. This is known from frequent anatomical and histological examination of snail gonads throughout the period extending between the cessation of spawning and the completion of gametogenesis. Three geographically separated populations were followed: (1) Barnstable Harbor, Cape Cod, Massachusetts; (2) Beaufort, North Carolina; and (3) Charleston, South Carolina, the latter two in somewhat less detail than the former. In the northern end of the range, gametogenesis usually proceeds within six weeks after the cessation of spawning, that is, sometime during late September. However, in the southern end of the range, spawning is completed by mid-June (Jenner 1956b), but the onset of gametogenesis is apparently delayed for several months. This delay needs confirmation by more frequent observations. There is no question, however, that both in New England and at Beaufort, North Carolina, gametogenesis has been completed by late fall, *i.e.,* mid- to late November. The attainment of sexual competence can

readily be determined externally in the living intact organisms by the structure of the penis in the male and by the pigmentation at the end of the oviduct in the female.

Natural spawning normally begins in February at the southern end of the species range, about mid- to end-April in Delaware Bay and the south shore of Cape Cod, and early June in Cape Cod Bay and Maine. Consequently, gametogenesis is completed almost six months before the natural spawning of populations found north of Cape Cod and at least two months before spawning in populations south of Cape Hatteras, North Carolina. Ecologically it is doubtlessly advantageous for the species to spawn as soon as the sea water becomes warm enough for larval development and the early gametogenesis allows great flexibility in the time of spawning.

The egg capsules of *N. obsoletus* are deposited on any solid object on the intertidal flats, *e.g.*, shells, *Diopatra* tubes, thallus algae, *etc.* Ankel (1929) has described in detail the deposition of egg capsules by the European species, *Nassarius reticulatus* L., and this account agrees in every essential detail with the process as it occurs in *N. obsoletus.* A description of the egg capsules of *N. obsoletus,* along with the characteristics distinguishing them from other members of the genus found along the east coast of the United States, has been given by Scheltema (1962a) and by Scheltema and Scheltema (1965).

Relationship of Temperature to Embryonic Development and the Escape of Larvae from the Egg Capsule into the Sea

The larvae of *Nassarius obsoletus* after the completion of their embryonic development emerge through an opening at the free end of the egg capsule. The precise method by which the opening is made by the larvae at the time of their escape is not understood, but its position at the distal end is structurally pre-determined at the time of capsule formation.

The relationship of temperature to the time required between spawning and emergence of veligers from their egg capsules can be demonstrated by a simple experiment. Adults of *N. obsoletus* readily lay egg capsules upon the sides of aquaria. If, shortly after their deposition, several hundred egg capsules are collected and placed at regular temperature intervals, falling within the extreme range at which they are normally found in nature, the effect of temperature on development can be determined. A number of such experiments were performed at temperature intervals of 28°, 19.5°, 16.5° and 11.5° C. Egg capsules laid within a 48-hour period were collected from snails that had been actively laying for several weeks. For the purpose of the experiment, the median age of the egg capsules was considered to be 24 hours. The exact time of deposition of each capsule is not particularly meaningful as the degree of development of the eggs within each capsule is known to vary at the time of attachment. Early in the spawning period, capsules are occasionally retained within the oviduct of the female until development of the embryos is almost completed to the veliger stage, but as the season of spawning proceeds there is normally little delay between the initiation of embryological development and egg capsule attachment. The rates of development at different temperatures were observed simultaneously from random aliquots taken from the same "harvest" of egg capsules. At the beginning of the experiment each capsule was examined to make certain that it was intact and had not been damaged during its removal from the walls of the aquarium. Between 250 and 300 egg capsules

were used at each temperature. Starting with the time at which larval emergence first began, the number of empty egg capsules in each container was determined at frequent intervals.

The results obtained in one such experiment are shown by the series of curves in Figure 1, in which the ordinate gives the cumulative percentage of capsules from which larvae had emerged and the abscissa the number of days since the deposition of the capsules. The curves represent development at each of the different temper-

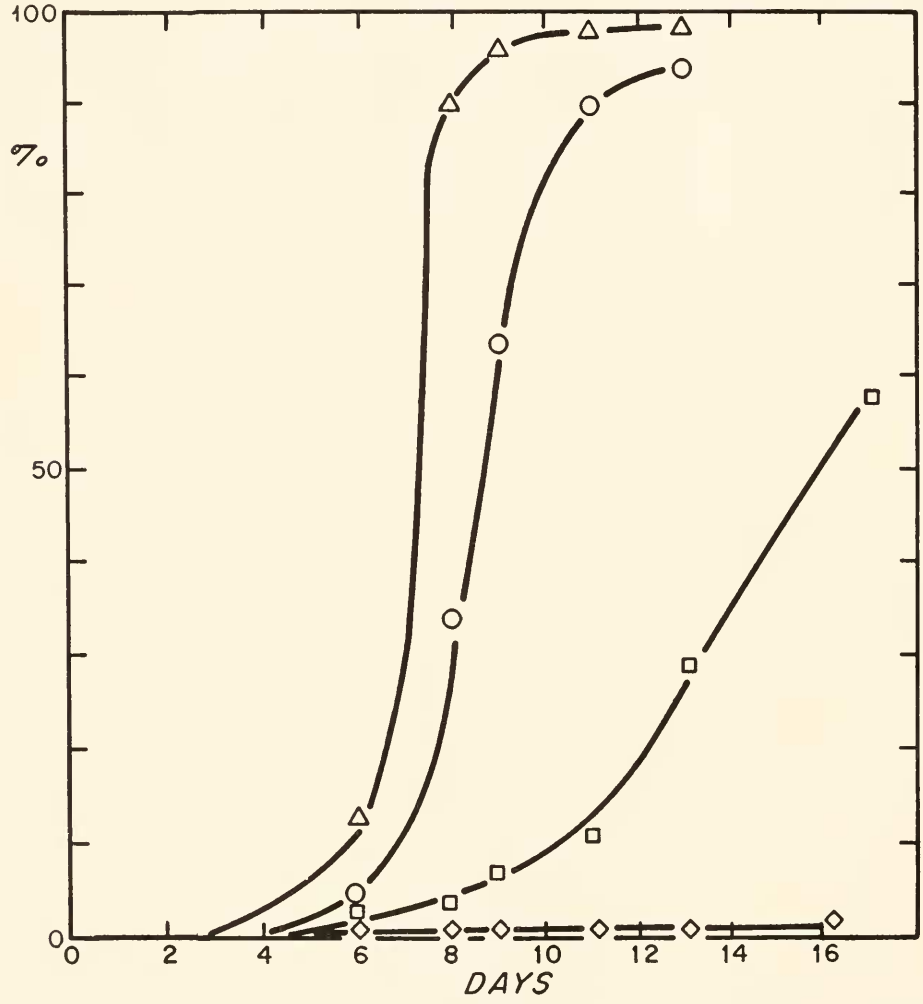

FIGURE 1. Percentage egg cases of *Nassarius obsoletus* from which larvae have emerged relative to the time since spawning occurred. The curves represent development at four different temperatures: 28.0° C. (△); 19.5° C. (○); 16.5° C. (□); and 11.5° C. (◇). The abscissa gives the time in days since the deposition of egg capsules; the ordinate is the cumulative percentage of egg capsules from which larvae have emerged. The values along the abscissa are approximate (± one day) as the egg capsules were laid over a 48-hour period.

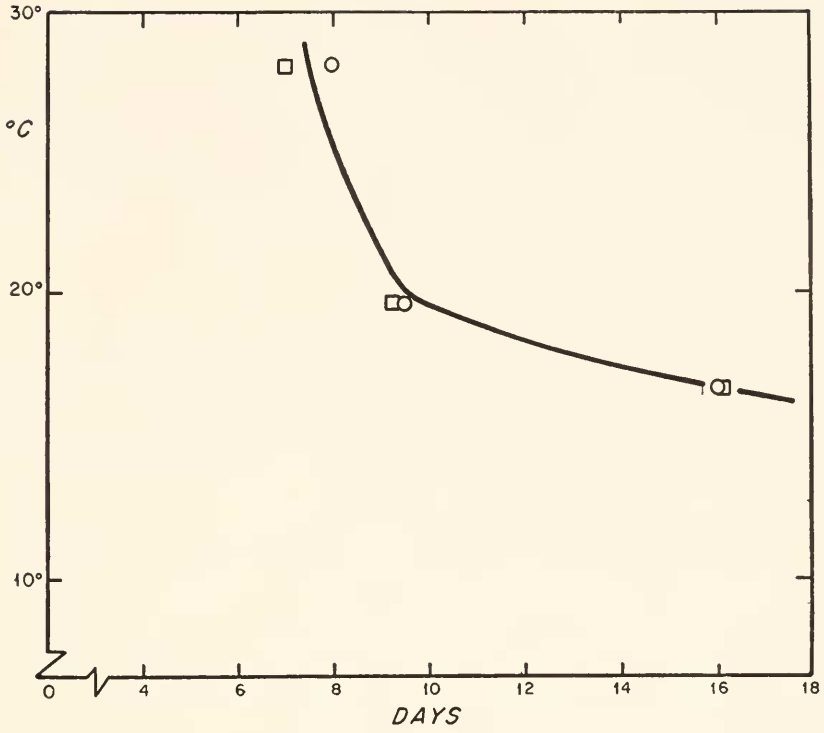

FIGURE 2. Time in days required between spawning and emergence of *Nassarius obsoletus* larvae from egg capsules as a function of temperature (° C.). The points indicate the number of days necessary for emergence of 50% of the larvae. Results are from two geographically isolated regions, Beaufort, North Carolina (○) and Cape Cod, Massachusetts (□). No significant difference is discernible in the results between egg capsules obtained from the two populations. The data are derived from experiments shown in Figure 1, and from similar experiments using egg capsules from snails obtained from Beaufort, North Carolina. From 250 to 300 egg capsules were used at each temperature in experiments with the Cape Cod populations. Approximately 100 egg capsules were used at each temperature in the Beaufort experiments.

atures. No larvae emerged from the capsules held at 11.5° C. during the course of the experiment. It has previously been shown that at a temperature between 11° and 13° C., embryos do not complete their development but that a large proportion remain viable for a period of up to at least nine weeks (Scheltema, 1962a). When returned to warmer water such embryos developed normally.

The relationship between temperature and the time required for the emergence of veliger larvae from the egg capsules is best understood by reference to Figure 2, where the number of days required for the liberation of veliger larvae from the first 50% of the capsules is indicated along the abscissa, and the temperature (° C.) at which the development took place is shown along the ordinate. Whereas the time required for emergence increases slightly between 28° and 20° C., about 0.25 day per degree centigrade, it increases more rapidly at temperatures below 20° C.; between 20° and 16.5° C., there was an increase of two days for each degree of

lowering of the temperature. The figure also shows that there is no significant difference in the effect of temperature on egg development in populations of snails from Beaufort, North Carolina, and from Cape Cod, Massachusetts. These results differ from those of Dehnel (1955) obtained from several intertidal species of gastropods along the west coast of North America. He found that when embryos collected from different geographical regions were allowed to cleave at an identical temperature, there was a clear difference in the developmental rate; the relationship appeared to be clinal.

The Relationship of Temperature to Growth Rate

A method for obtaining large numbers of *Nassarius obsoletus* veliger larvae and for growing mass cultures to be used in experimental work has already been described (Scheltema, 1962a). The cultures used in the present experiments were 10 liters in volume, each containing from 5000 to 10,000 larvae. Food used throughout the duration of these experiments was *Phaeodactylum tricornutum* Bohlin which was obtained from unialgal cultures.

Larvae which had emerged from a large number of egg capsules over a 24-hour period were divided equally among 10-liter containers. The number of larval cultures started was determined by the number of temperatures at which growth was to be measured. A sample of the larvae was also taken at the beginning of each experiment so that the initial size after emergence from the egg capsule could be determined. Each larval culture was fed an identical quantity of food (*ca.* 200,000 cells/ml.) from the same unialgal culture of *P. tricornutum*. This amount of food was great enough so that a slight excess remained after three days.

A sample of from 50 to 100 larvae was removed from each culture every third day. At this time the water was also changed and new food cells were added. The aliquot of larvae was preserved in 70% alcohol for later measurement.

The growth of larvae was estimated by measuring the shell length of 35 specimens picked randomly from the larger aliquot described above. An ocular micrometer at a magnification of 100 × was used. The longest dimension of the shell of a larva was considered to be the length.

The temperature of the cultures was maintained by means of water baths improvised from commercial soft drink coolers. The maximum deviation from the stated mean was 1.5° C., but the mean deviation was only ±0.5° C. Because all the experiments extended over more than two weeks, these variations were not considered serious.

In the first series of experiments, larvae were grown simultaneously at either three or four different temperatures. The results from one representative experiment are shown in Figure 3, where the mean temperatures were 16.5°, 21.0°, 24.8° and 29.5° C. From this experiment it was concluded that the optimum temperature for growth under laboratory conditions was approximately 25° C. This was further verified in three other experiments. At either higher or lower temperatures the growth was significantly less. That the rate of growth is not uniform throughout larval development, particularly at optimum temperatures, can also be seen in Figure 3. The lowest temperature at which larvae successfully grew to metamorphosis was between 16° and 17° C.

To determine the maximum effect of temperature upon growth I made a second series of experiments. The growth rate at approximately 25° C., an optimum temperature, was compared with that at 17.5° C., a value near the lowest temperature at which larval development is completed to settlement. The results of one such series of experiments are shown in the growth curves in Figure 4. Here the upper cumulative growth curve is from larvae grown at 25.2° C.; the lower curve represents growth under similar conditions except that the temperature was 17.5° C. The minimum length at which the veliger larvae have been shown to metamorphose

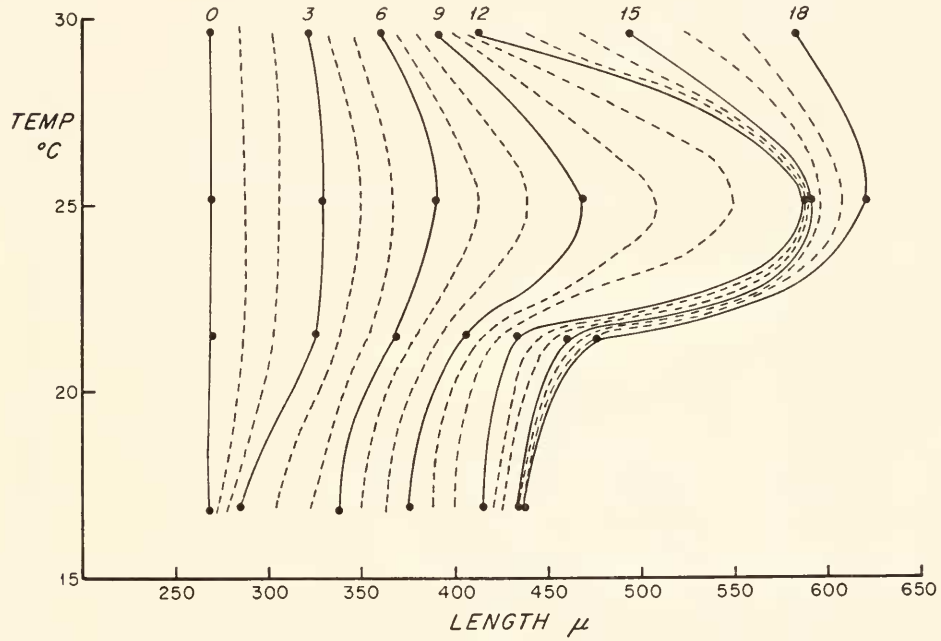

FIGURE 3. The effect of temperature upon the growth of the planktonic veliger larvae of *Nassarius obsoletus*. The ordinate gives the temperature range over which the larvae were grown. The abscissa gives the length attained by the larvae. Each curve shown by a solid line represents the total cumulative growth completed by the larvae within the number of days indicated by the numeral over the curve. The amount of growth for any length of time and for any temperature which was tested can be easily derived from the figure. The points on the solid lines represent the actual experimental values obtained. Curves given with dashed lines represent the average growth at intervening days and were derived by linear interpolation.

lies between 550 and 600 μ, but the median size is near 700 μ. On the graph in Figure 4 the inflection points on both curves are at approximately 600 μ. In order to compare growth rates between two temperatures it is clearly necessary to consider only those portions of the curves which precede the points of inflection. After the median size for metamorphosis is reached (*i.e.*, 700 μ) relatively little further growth occurs. The maximum recorded size at metamorphosis is 950 μ, but this size is rarely attained by larvae. The length of the period following the completion

of development (*i.e.*, the attainment of 700 μ) is primarily dependent on a settlement response of the larvae. This is further discussed below.

All the experiments, including those of the first series above for which no data have thus far been given, are summarized in Table I. The data from all these

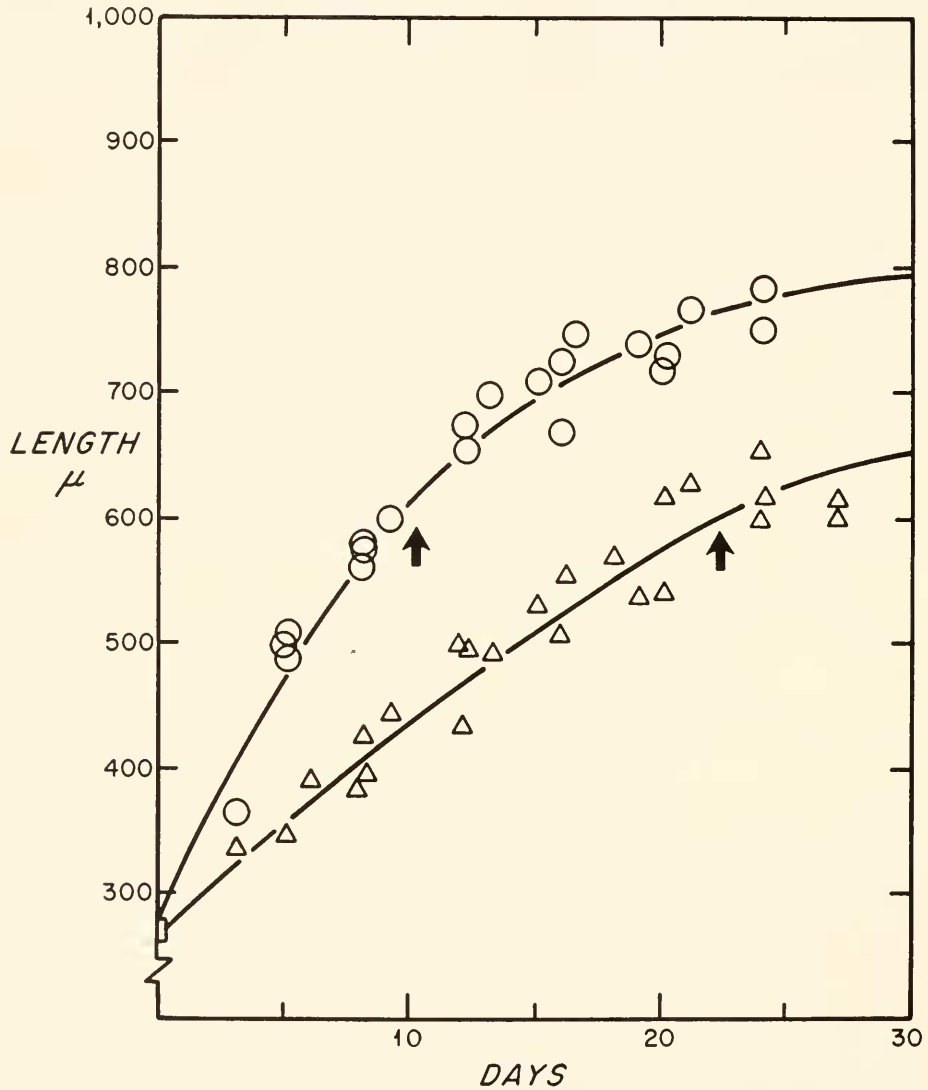

FIGURE 4. Cumulative growth curves of the planktonic veliger larvae of *Nassarius obsoletus*. The upper curve (○) represents cumulative growth at an average temperature of 25.2° C.; the lower curve (△) represents growth of larvae at 17.5° C. The attainment of the "creeping-swimming" stage is indicated on each curve by an arrow. This shows the end of the "developmental period" to the left of arrow and the beginning of the "delay period" to the right of arrow. Note that the "developmental period" is approximately twice as long at 17.5° C. (21 days) as it is at 25.2° C. (10 days).

experiments cannot be directly compared because (1) the larvae were not randomly obtained from the same parents and therefore are not known to be genetically similar, and (2) the experiments were not conducted simultaneously, using the same algal culture, so that the quality of the food was not necessarily the same. However, the data can be pooled and the results compared by using the value I which is the *per cent inhibition of growth* (Scheltema, 1965). This may be computed from the equation

$$I = \frac{\Delta A - \Delta B}{\Delta A} \times 100$$

where ΔA is the change in length of the shell between the beginning and end of an experiment at a temperature optimum for growth (*ca.* 25° C.) and ΔB is the

TABLE I

The growth of Nassarius obsoletus larvae at a near optimal temperature and at a minimum temperature required for completion of development, showing the maximum inhibition attributable to temperature

Expt. no.	Age in days at end of expt.	Length μ at begin. expt. (a)	Mean temp. °C.	Length** μ at end expt. (A)	Growth μ (ΔA)	Mean temp. °C.	Length** μ at end expt. (B)	Growth μ (ΔB)	Per cent inhibition of growth $\frac{\Delta A - \Delta B}{A} \times 100$
I	12	280*	25.1	657± 6	377	17.3	436±5	156	59
II	12	280*	25.0	655± 8	375	17.2	498±8	218	42
III	13	280*	24.5	698±10	418	17.7	488±7	209	50
IV	12	268	26.4	569± 5	301	17.5	496±5	228	24
V	12	268	26.4	672± 7	404	17.3	492±5	234	42
VI	17	271	23.9	615±11	344	15.8	447±7	176	49
VII	12	262	23.8	529± 7	267	15.9	422±8	160	40
VIII	9	268	24.6	463± 5	195	16.5	361±5	93	52
IX	12	268	25.3	589± 7	321	16.6	413±6	145	55
									mean = 46

* Estimated values.
** One standard error of the mean is indicated.

change in shell length at a minimum temperature required for the completion of development.

The value of ΔA is determined by subtracting the initial length of the veliger larvae at the beginning of the experiment from the length attained when the experiment was terminated. Hence

$$\Delta A = (A - a)$$

where a is the initial length at the time the larvae emerged from the egg capsule and A is the final length of the larvae when grown at 25° C. Similarly ΔB is obtained by subtracting the initial length a, from B where B is the final length of the larvae grown at around 16° or 17° C. The time at which each experiment was terminated was determined by the inflection point on that curve which represented the culture having optimum growth (*i.e.*, 25° C.).

With a single exception the values of I fall between 40% and 60% and the mean *per cent inhibition of growth* attributable to temperature was approximately 46%. This represents the average maximum-difference which can be accounted for by temperature alone.

Discussion

As the onset of spawning by *Nassarius obsoletus* is dependent upon temperature, its timing is never precise. Gametogenesis is always completed several months before spawning occurs, and consequently a short period of warming can very easily initiate spawning. Such conditions occur when the low tide falls near noon on clear sunny days during early spring. Experimental evidence now shows, however, that embryos can survive over long periods in cold water, but at a sharply reduced developmental rate. Somewhat similar results have been obtained with *Nassarius reticulatus* from the Black Sea (Bekman, 1941). It is very unlikely, from experimental evidence, that embryonic development of *N. obsoletus* into free-swimming veliger larvae is ever completed, under conditions in nature, before the water temperature rises high enough to insure the completion of pelagic development.

Although an optimum growth of planktonic larvae in the experiments occurred at 25° C., it is not clear whether this was an intrinsic characteristic of the veligers themselves or whether growth was indirectly influenced by the effect of temperature on the algal food. *Phaeodactylum tricornutum* does not long survive at temperatures above 25° C. However, as the larvae were fed fresh algal cells every third day and since an excess always remained in suspension at the end of this period, it was believed that this effect must have been minimal. It is not possible, however, to rule out such an indirect factor in the experimental results. Davis and Calabrese (1964) have suggested that enzymes required to digest naked flagellates are active at much lower temperatures than those involved in the digestion of certain other food forms having thick cell walls. Very few dinoflagellates or diatoms can grow and survive equally well at temperatures of 15° and 30° C.; both their numbers and food value to larvae may differ markedly at either of these extremes. It is necessary, when relating experiments from the laboratory to natural conditions, to take into account the effect of temperature on the principal phytoplankton organisms upon which the larvae are likely to be feeding.

The length of pelagic larval life in some bivalves seems to be directly related to the temperature and growth rate. Thus Loosanoff (1959) has shown that the increase in pelagic larval life of *Venus mercenaria* is directly related to the decrease in temperature. However, the results are somewhat obscured because the criterion used to determine the length of larval life was the number of days required for settlement to first begin. Such a criterion largely neglects the effect of a delay in settling due to the lack of a desirable substratum, if indeed *Venus mercenaria* has such a delay. Davis and Calabrese (1964, p. 648) have shown that in *Crassostrea virginica* the last larvae to settle in their cultures usually have a planktonic life almost two times as long as the earliest veligers to metamorphose. Bayne (1965) has demonstrated that *Mytilus edulis* in the absence of an adequate substratum for settlement delays metamorphosis, and that this delay is accompanied by a gradual decrease in growth rate to zero.

The larval life of *Nassarius obsoletus* can be divided into two periods. The

first of these is one of rapid growth and morphological development and will be termed the "developmental period." This is followed by a second period, the "delay period," during which there is a gradual decrease in growth. The "developmental period" ends at the inflection point on the cumulative growth curve (Fig. 4). External morphological development has been completed to the creeping-swimming stage (Scheltema, 1962a). The growth rate during the "developmental period" is essentially constant if the environment remains reasonably so. Two physical factors that are important in determining the slope of the growth curve, and consequently the length of the "developmental period." are temperature and, under certain circumstances, the salinity of sea water (Scheltema, 1965). At the end of the "developmental period," metamorphosis first becomes possible. The "delay period" which follows may vary greatly in its length. Its duration is largely determined by the availability of the bottom sediment favorable for further post-larval life. The evidence for delay in settlement and a response to bottom sediment in *N. obsoletus* has already been given in previous papers (Scheltema, 1956, 1961).

Summary

1. Development of the embryos of *Nassarius obsoletus* within egg capsules is regulated by sea-water temperature. An increase in the time required between spawning and the emergence of veliger larvae is slight between 28° and 20° C., about 0.25 day for each degree decrease in temperature. Between 20° and 16.5° C., the corresponding increase was 2 days per degree decrease in temperature. At 11.5° C., development was not completed and larvae did not emerge from their egg capsules after nine weeks. However, a large proportion of these embryos survived and developed normally through metamorphosis when placed at room temperature.

2. The growth rate of planktonic veliger larvae of *N. obsoletus* was greatest at approximately 25° C. The lowest temperature at which the development to metamorphosis was completed was at 16° to 17° C. There was a 46% inhibition in the growth rate of larvae between the optimum temperature and the minimum temperature at which development is completed.

3. The larval life of *N. obsoletus* veligers may be divided into two stages. The first of these, the "developmental period," is one during which rapid growth and morphological development occur. This is followed by the "delay period" characterized by a gradual decrease in growth rate. Reduced temperature may influence the rate of growth and consequently the length of the "developmental period." The termination of the "developmental period" comes with the "creeping-swimming stage." The duration of the "delay period" may be quite variable and is determined by the availability of a favorable sediment for settlement.

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